SHORTER ARTICLES AND DISCUSSION

PIEBALD RATS AND MULTIPLE FACTORS

IN the NATURALIST for December, 1916, MacDowell has published an extended criticism of experiments in the modification of the hooded pattern of piebald rats by selection, in which my colleagues and I have been engaged for some years. This is not the first time that readers of the NATURALIST have had their attention called to these experiments by similar adverse criticism and they are possibly quite weary of the subject. In so far as MacDowell merely offers in new form arguments which have already been presented by Muller and Pearl and answered by me, I shall make no reply. But as regards two points which may fairly be considered critical, one of which actually is so designated by MacDowell, I desire to present some evidence which I regard as conclusive but which MacDowell has not discussed, evidence possibly not accessible to many readers of this journal. MacDowell's criticism is based on the data presented in Publication 195 of the Carnegie Institution (Castle and Phillips, 1914), and in a brief paper in *The Scientific Monthly* (1916). Many additional data are given in Publication 241 of the Carnegie Institution, but this is not considered by Mac-Dowell, although it was issued in September, 1916, as Paper No. 26 of the station with which he is connected, nearly two months in advance of his own paper. Had he considered carefully the evidence contained in this later publication, I am sure that he would have modified his criticism materially.

In 1914 Phillips and I offered two alternative explanations of the progressive changes observed under selection in the hooded pattern. These were (*a*) variability of the unit-character ("factor") hooded, and (*b*) multiple modifying factors affecting the hooded character. We found it difficult to decide between these two interpretations on the basis of evidence then available. For this hesitancy we were promptly taken to task by Muller, who championed the multiple factor interpretation now adopted also by MacDowell. MacDowell elaborates in much detail a dozen points which show compatibility between our observations and a multiple factor hypothesis, but without consid-

ering whether they are also compatible with the alternative hypothesis of a single varying factor. Modification on crossing, decreasing variability, regression, greater variability in F_2 than in F_1, effective return selection—these are all phenomena to be expected equally on either hypothesis. To cite them is no argument for one hypothesis rather than the other. This point has wholly escaped both Muller and MacDowell, who seem quite unable to conceive any but the single explanatory principle of multiple factors.

Putting aside these irrelevant arguments, there remain two points in MacDowell's discussion which require further consideration. They are the same two points which led us in 1914 to hesitate between the alternative interpretations which we considered, but on which we now have fuller evidence.

But before we go into this new evidence one or two minor points may be noted in which the accuracy of our generalizations is questioned. MacDowell has gone over our 1914 publication in great detail, devoting as many pages to its destructive criticism as we to its original exposition, and recalculating the statistical coefficients. It is gratifying to know that he has detected in these no serious errors, though his figures differ from ours slightly in some cases. Whether his calculations are more accurate than our twice-checked ones, I am unable to say without detailed reexamination of the data. As these are public property, the curious reader may satisfy himself on the point. I do not consider it necessary to go into the matter anew since the substantial correctness of our findings is not challenged.

MacDowell thinks that we did not sufficiently emphasize the decreasing variability (standard deviation) and the decreasing rate of divergence of the selected races, observed as the selection progressed. These to his mind imply that selection would sooner or later cease to be effective. In this opinion I can not concur, since in neither the plus nor the minus selection series has the standard deviation decreased by half, although sixteen successive selections had been made and the hooded character had been so modified as to be scarcely recognizable longer. Whether one considers the decrease in variability large or small depends principally upon how much importance he attaches to the values found for the first two generations of the experiment, when the numbers of individuals observed were still small and methods of grading them had not yet been fully standardized. Mac-

Dowell emphasizes the high variability of these early generations, few in individuals, and attaches importance to the *relatively* smaller variability of later generations. It seems to me fairer to compare the first half of the series with the second half. Concerning the point I have said (Publication 241, p. 172):

> The amount of the variation as measured by the standard deviation is less in the last half of the experiment than in the first half. It is also *steadier*, owing in part doubtless to the fact that the numbers are larger, and in part to a more stable genetic character of the selected races. But the genetic variability is plainly still large enough to permit further racial modification and there is no indication that it will cease until the hooded character has been completely selected out of existence, producing at one extreme of the series all-black rats, and at the other end of the series black-eyed white rats.

It should be noted that these conditions have already been approximated in individual cases.

THE NEW EVIDENCE

1. *The progeny of plus selected crossed with wild rats.* (Quoted without change from Publication 241, pp. 163–168.)

In 1914 Castle and Phillips published a report on breeding experiments with hooded rats, in which it was shown that the hooded color-pattern—itself a Mendelian recessive character in crosses with the entirely colored (or " self ") coat of wild rats—is subject to quantitative variation, and that different quantitative conditions of the hooded pattern are heritable. (Compare fig. 36, plate 7.) It was also shown that by repeated selection of the more extreme variations in the hooded pattern (either plus or minus) it is possible gradually to modify the racial mean, mode and range as regards these fluctuations, without eliminating further fluctuation or greatly reducing its amount. We concluded that the unit-character, hooded color-pattern, is a quantitatively varying one, but were at that time unable to decide whether the observed variability was due simply and exclusively to variation in a single Mendelian unit-factor or partly to independent and subsidiary modifying Mendelian factors.

Since publication of the above I have been engaged in further experiments designed to show which of the alternative explanations is the correct one, and these are now sufficiently advanced to indicate definite conclusions. Previous experiments had shown that when a race of hooded rats, whose character has been modified by selection (either plus or minus), is crossed with wild rats, the extracted hooded animals obtained in F_2 as recessives show regression toward the mean condition

of the recessive race before selection began. This result suggested that the regression observed might be due to removal by the cross of modifying factors, which selection had accumulated in the hooded race. If this view was correct, it was thought that further crossing of the extracted hooded animals with the same wild race should result in *further* regression, and that if this further regression was *not* observed a different explanation must be sought for the regression already noted.

The entire experiment has accordingly been repeated from the beginning, with the same result as regards regression in the first F_2 generation, but with *no regression* of the same sort in a second F_2 containing twice-extracted hooded animals. So far from observing further regression as a result of the second cross with wild rats, we have unmistakable evidence that the movement of the mean, mode and range of the hooded character has been in the *reverse* direction. So the hypothesis of modifying factors to account for the regression and for the progressive changes observed under selection becomes untenable.

In repeating the experiment of crossing hooded rats of our selected races with wild rats, great care has been taken to employ as parents individuals of the greatest racial purity and to inbreed the offspring brother with sister, thus precluding the possibility of introducing modifying factors from other sources. In making the second set of crosses, the extracted individual has, wherever possible, been crossed with its own wild grandparent. In the few cases in which this was impossible, wild animals of the same stock have been used. This stock consisted of a colony of wild rats which invaded the basement of the Bussey Institution apparently from a near-by stable. Owing to faulty construction of the building they were able to breed in spots inaccessible to us, and it took many months of continuous and persistent trapping to secure their extermination. During this period we trapped a hundred or more of them, all typical Norway rats, colored all over, without even the white spot occasionally seen on the chest of wild rats. Two generations of rats from this wild stock have been reared in the laboratory, and all have this same self-colored condition.

The hooded animals used in the experiments to be reported on in this connection consisted of 4 individuals of the plus selected series, a male and 3 females, as follows:

TABLE 140

Individual.	Grade.[1]	Generation.
♀ 5513.....	$+4\frac{1}{4}$	10
♂ 6348.....	$+4$	10
♀ 6600.....	$+4\frac{1}{4}$	12
♀ 6955.....	$+4$	12

[1] See figure 35, plate 7 for significance of the grades.

Each of these animals was mated with a single wild mate, and their children were weaned directly into breeding cages containing a male and two or three females (brother and sisters). In the case of two matings, F_1 males of the same parentage were at the time lacking and males from a different cross were used. The results of such matings are tabulated by themselves and serve a useful purpose as controls. The F_1 animals all closely resembled their wild parents, but many of them had a white spot on the chest. They ranged from grade $+5\frac{1}{4}$ to $+6$ (self).

The F_2 animals are classified in table 141, where it appears that 73 of them were hooded and 219 non-hooded (i. e., like F_1), an exact 1:3 ratio. More than half of this F_2 generation consists of the grand-

TABLE 141

Table 141 shows the classification of extracted hooded *first* F_2 young obtained from crossing hooded rats of the plus-selected series with wild rats.

Hooded grandparents.	Grade of hooded grandchildren.											Total hooded.	Total non-hooded.	Means of hooded.
	$1\frac{1}{2}$	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4			
♀ 5513, $+4\frac{1}{4}$, gen. 10....	1	..	3	2	1	7	8	6	5	7	1	41	107	3.05
♂ 6348, $+4$, gen. 10.....	..	..	1	..	1	2	4	3	4	6	1	22	68	3.28
♀ 6955, $+4$, gen. 12.....	..	..	..	..	..	..	..	1	1	3	..	5	27	3.51
♀ 5513, $+4\frac{1}{4}$, and ♀ 6600, $+4\frac{1}{4}$, gen. 12....	..	..	..	..	..	..	2	..	1	..	..	3	12	3.17
♀ 5513, $+4\frac{1}{4}$, and ♀ 6955, $+4$, gen. 12.....	..	..	..	..	..	..	..	1	1	..	..	2	5	3.37
Totals.............	1	..	4	2	2	9	14	11	12	16	2	73	219	3.17

children of ♀ 5513, produced by breeding her children brother with sister, those children all having been sired by the same wild rat. Her grandchildren include 41 hooded and 107 non-hooded young. The hooded young range in grade from $+1\frac{1}{2}$ to $+4$, their mean grade being $+3.05$, a considerable regression from the grade of the grandmother, which was 4.25.

Hooded rats of the same grade and generation as the grandmother, when bred with each other, produced young of mean grade $+3.84$. (See table 10, Castle and Phillips.[2]) The mean of the extracted hooded grandchildren in this case (being 3.05) shows a regression of 0.79 from that expected for the uncrossed hooded race. From the extracted hooded grandchildren of ♀ 5513, produced as just described by a cross with a wild male, 7 individuals, 2 males and 5 females, were selected for a second cross with the wild race. They ranged in grade

[2] Comparison should have been made with generation 11 offspring, whose mean was 3.91 not 3.84. This would make the regression 0.86, instead of 0.79.

TABLE 142

Table 142 shows the classification of extracted hooded *second* F_2 young obtained from crossing first F_2 hooded rats (table 141) with wild rats. The hooded grandparents were themselves grandchildren of ♀ 5513, $+ 4\frac{1}{4}$, generation 10, on the side of both parents.

Hooded grandparents.	Grade of hooded grandchildren.									Total hooded.	Total non-hooded.	Means of hooded.
	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4			
♀ 9619, $+ 2$........	...	...	...	...	...	1	1	...	...	2	8	3.37
♂ 9686, $+ 2\frac{3}{4}$......	...	...	...	...	1	1	2	1	...	5	28	3.40
♀ 9620, $+ 2\frac{3}{4}$......	1	1	2	1	1	2	2	3	...	13	24	3.06
♀ 9729, $+ 2\frac{3}{4}$......	...	...	...	...	...	1	4	4	1	10	22	3.62
♂ 9727, $+ 3$........	...	1	...	2	3	2	11	7	4	30	104	3.47
♀ 9728, $+ 3$........	...	...	1	...	1	5	6	8	1	22	68	3.55
♀ 9621, $+ 3\frac{1}{4}$......	...	...	...	1	...	1	2	7	5	16	42	3.70
Totals...........	1	2	3	4	6	13	28	30	11	98	296	3.47

from $+ 2$ to $+ 3\frac{1}{4}$. (See table 142.) They produced several litters of young of the same character as the first F_1 young, all being similar to wild rats in appearance, except for the frequent occurrence of a white spot on the belly. These second F_1 young were at weaning time mated, brother with sister, in breeding-pens, precisely as had been done with the first F_1's. They produced 394 second F_2 young, of which 98 were hooded and 296 non-hooded, a perfect 1:3 ratio. The hooded young varied in grade from $+ 2$ to $+ 4$, as shown in table 142, the data there being given for each family separately as well as for all combined in the totals. One family was very like another as regards the character of the hooded young, except that the higher-grade grandparents had grandchildren of slightly higher grade. Thus the average of all the 98 hooded young was $+ 3.47$, but the average of those descended from the 3 grandparents of lowest grade was *less* than this, while the average of those descended from the 3 grandparents of highest grade was *greater*. This is just what had been observed throughout the entire selection experiments. (See Castle and Phillips.)

If we weight each of the grandparents in table 142 in proportion to the number of its hooded grandchildren, then the mean grade of all the grandparents is $+ 2.95$. Since the mean grade of all the 41 first F_2 hooded grandchildren, from which these 7 were chosen, was $+ 3.05$, it will be seen that these 7 are, so far as grade is concerned, fair representatives of the 41, being in fact of slightly lower mean grade. It is therefore all the more striking that their grandchildren, the second F_2 hooded young (table 142), are of higher grade. They regress in an *opposite* direction to that taken by the first F_2 hooded young. Thus the original hooded ancestor (♀ 5513) was of grade 4.25. The grade of hooded young expected from such animals is 3.84. What she produced

in F_2 following a cross with the wild male, was young of mean grade 3.05. Seven of these of mean grade 2.95 produced a second F_2 containing hooded young of mean grade 3.47. This is a *reversed* regression of 0.52 on the grade of their actual hooded grandparents, or of 0.42 on the group from which their grandparents were chosen. Their mean lies about midway[3] between that which would have been expected from the original hooded female (5513) had no crossing with wild rats occurred and that which was observed in the first F_2.

Obviously these facts do not harmonize with the assumption that the regression observed in the first F_2 was due to loss of modifying factors accumulated during the ten preceding generations of selection; for no further loss occurs in the second F_2. On the other hand, a partial recovery is made of what was lost in the first F_2. This suggests the idea that that loss may have been due to physiological causes nongenetic in character, such as produce increased size in racial crosses; for among guinea-pigs (as among certain plants) it has been found that F_1 has an increased size due to vigor produced by crossing and not due to heredity at all. This increased size persists *partially* in F_2, but for the most part is not in evidence beyond F_1. I would not suggest that the present case is parallel with this, but it seems quite possible that similar non-genetic agencies are concerned in the striking regression of the first F_2 and the subsequent reversed regression in the second F_2.

Whatever its correct explanation may be, the *fact* of the reversed regression in a second F_2 is very clear, as other cases than those already discussed will show.

A hooded rat of grade $+4$ and generation 10, ♂ 6348, had by a wild female several young of the character already described for the young of ♀ 5513. These, mated brother with sister, produced a first F_2 (table 141) of 90 rats, 22 of which were hooded, 68 being non-hooded, again a good $1:3$ ratio. The hooded young ranged from $+2$ to $+4$ in grade, their mean being 3.28. Of the 22 hooded individuals, 1 male and 7 females were mated with wild rats to obtain a second F_1, and the second F_1 animals were then mated brother with sister to obtain the desired second F_2. The character of this is shown family by family in table 143. It contained 497 individuals, of which 121 were hooded and 376 non-hooded, a ratio of $1:3.1$. The weighted mean of the 8 selected grandparents is 2.93, which is 0.35 below the mean of the 22 first F_2 hooded animals which they represent. The mean of the second F_2 hooded young is 3.22, which indicates a *reversed* regression of 0.29

[3] In *The Scientific Monthly* (Jan. 1916) I have stated that a second cross showed ''a return to about what the selected race would have been had no crossing at all occurred.'' This is obviously inaccurate and should be corrected. It rests on a comparison with the combined average of both the older and the more recent experiments. [MacDowell devotes half a page to demolishing the statement already corrected here.]

on the grade of the grandparents, but shows no significant difference from the mean of the grandparental group (3.28).

TABLE 143

Table 143 shows the classification of extracted hooded *second* F_2 young obtained from crossing first F_2 hooded rats (table 141) with wild rats. The hooded grandparents were themselves grandchildren of $\male$ 6348, $+4$, generation 10, on the side of both parents.

Hooded grandparents.	Grade of hooded grandchildren.										Total hooded.	Total non-hooded.	Means of hooded
	$1\frac{3}{4}$	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4			
$\male$ 9639, $+2$.....	1	2	1	...	3	6	4	15	6	1	39	110	3.24
$\female$ 9704, $+2\frac{3}{4}$....	...	1	...	...	...	1	...	4	...	...	6	16	3.17
$\female$ 9765, $+3$.....	...	...	...	...	...	1	...	...	...	...	1	10	3.50
$\female$ 9747, $+3\frac{1}{4}$....	1	7	...	1	1	7	1	4	4	1	27	76	2.90
$\female$ 9703, $+3\frac{1}{2}$....	...	...	1	1	2	2	1	5	2	2	16	47	3.28
$\female$ 9705, $+3\frac{1}{2}$....	...	...	...	...	1	5	1	4	8	2	21	74	3.48
$\female$ 9748, $+3\frac{1}{3}$....	...	...	...	...	1	2	1	2	3	...	9	40	3.36
$\female$ 9796, $+4$.....	...	...	...	...	...	...	...	...	1	1	2	3	3.87
Totals........	2	10	2	2	8	23	8	35	24	7	121	376	3.22

All except one of the 8 families classified in table 143 show unmistakably the reversed regression. This exceptional family consists of the grandchildren of $\female$ 9747. They have a mean grade of 2.90, substantially the same as that of the entire group of grandparents, but considerably lower than that of their own hooded grandmother. Apparently she did not come up genetically to her phenotypic grade. This the other grandparents of the group did. For those of lowest grade (2, $2\frac{3}{4}$) produced lower-grade hooded grandchildren than did the grandparents of highest grade ($3\frac{1}{2}$, 4), as was found to be the case also in table 142.

We may next trace the inheritance of the hooded character through a third but smaller family produced by two successive crosses with wild rats, the hooded character in this case being derived from $\female$ 6995, grade $+4$, generation 12. The character of her first F_2 descendants is shown in table 141. They consist of 5 hooded and 27 non-hooded individuals. The mean grade of the hooded young is 3.51, but the number of these young is too small to make this mean of much significance. One of the hooded young ($\male$ 9660, $+3\frac{3}{4}$) was mated with a wild female to secure a second F_1 generation and from this in due course was produced the second F_2 generation (table 144). It consisted of 21 hooded and 44 non-hooded young. The hooded young showed the usual range (2 to 4). Their mean grade was 3.50, substantially identical with that of the first F_2 animals, but 0.25 below that of the actual hooded grandparent. This family history is less satisfactory than the two already discussed because of the smaller numbers which it includes. It

contains nothing contradictory to the interpretation already given, though reversed regression is not in this case in evidence.

TABLE 144

Table 144 shows the classification of extracted hooded *second* F_2 young obtained from crossing first F_2 hooded rats with wild rats. The hooded grandparent, ♂ 9660, $+ 3\frac{3}{4}$, was a grandson of ♀ 6955, $+ 4$, generation 12, on the side of both parents. The hooded grandparent ♂ 9711, $+ 3\frac{1}{4}$, was a grandson, on the side of one parent, of ♀ 5513, $+ 4\frac{1}{4}$, generation 10, and on the side of the other parent, of ♀ 6955, $+ 4$, generation 12. (See table 141.)

Hooded grandparents.	Grade of hooded grandchildren.									Total hooded.	Total non-hooded.	Means of hooded.
	2	$2\frac{1}{4}$	$2\frac{1}{2}$	$2\frac{3}{4}$	3	$3\frac{1}{4}$	$3\frac{1}{2}$	$3\frac{3}{4}$	4			
♂ 9660, $+ 3\frac{3}{4}$.......	1	...	...	1	1	2	5	9	2	21	44	3.50
♂ 9711, $+ 3\frac{1}{4}$.......	...	...	1	2	2	4	4	2	1	16	33	3.28
Totals...........	1	...	1	3	3	6	9	11	3	37	77	3.40

In two cases F_1 females could not be mated with brothers and so mates were taken from other families. Thus " mixed F_1 matings " were made between children of 5513 and 6600 and children of 5513 and 6955. (See table 141.) The former mating produced 3 hooded and 12 non-hooded " first " F_2 young; the latter produced 2 hooded and 5 non-hooded " first " F_2 young. The grade of the hooded young produced by these mixed matings was not different from that of brother-sister matings, so far as the small numbers permit one to judge. One of these mixed matings was carried into a second F_2 generation. The first F_2 hooded ♂ 9711, $+ 3\frac{1}{4}$, was mated with a wild female, and the young were bred, brother with sister, producing 16 hooded and 33 non-hooded young. (See table 144.) The mean grade of the 16 hooded young was 3.28, substantially the same as that of the first F_2 hooded grandparent. No additional regression through loss of modifiers (or other agency) is here in evidence. The result is the same as that observed in families wholly unmixed. The attention of my pure-line critics, who think that in our mass selection experiments insufficient attention has been given to individual pedigrees, is particularly directed to the foregoing case.

Having now discussed each family history separately, we may combine all the second F_2 families in one table, in order to get a clearer impression of the results as a whole. (See table 145.) The second F_2 generation thus combined includes 256 hooded and 749 non-hooded individuals, a ratio of 1 : 2.9, an unmistakable mono-hybrid Mendelian ratio. The mean grade of the hooded individuals is 3.34. The weighted mean grade of their hooded grandparents was 3.02, which indicates a *reversed regression* of 0.32 for the entire second F_2 group of hooded animals.

TABLE 145

Table 145 is a combination of tables 142 to 144, in which the second F_2 young are classified according to the grade of their first F_2 hooded grandparent.

Grade of hooded grandparents.	Grade of hooded grandchildren.										Total hooded.	Total non-hooded.	Means of hooded.
	1¾	2	2¼	2½	2¾	3	3¼	3½	3¾	4			
2	1	2	1	...	3	6	5	16	6	1	41	118	3.25
2¾	...	2	1	2	1	3	4	12	8	1	34	90	3.29
3	...	...	1	1	2	4	7	18	15	5	53	182	3.48
3¼	1	7	...	2	4	9	6	10	13	7	59	151	3.22
3½	...	...	1	1	4	9	3	11	13	4	46	161	3.39
3¾	...	1	...	...	1	1	2	5	9	2	21	44	3.50
4	...	...	...	...	...	...	...	...	1	1	2	3	3.87
3.02	2	12	4	6	15	32	27	72	65	21	256	749	3.34

Classified according to the grade of the (first F_2) grandparent, they show a correlation between grade of grandparent and grade of grandchild. The lower-grade grandparent has lower-grade hooded grandchildren, and the higher-grade grandparent has higher-grade hooded grandchildren. This shows that the variation in grade is (in part at least) *genotypic*. As the experiment yields no evidence that the variation in the hooded character is due to independent modifying factors, there remains no alternative to the conclusion that the single genetic Mendelian factor concerned fluctuates in genetic value. Fluctuation accordingly is not exclusively phenotypic, as DeVries and Johannsen have thought, but may be genetic also. Hence racial changes may be effected through selection by the isolation of genetic fluctuations, as well as by the isolation of mutations. Moreover, genetic fluctuation makes possible *progressive change* in a particular direction, repeated selection attaining results which it would be quite hopeless to seek by any other means.

2. *The progeny of "mutant" crossed with wild rats.*   (From Publication 241, p. 173.)

Castle and Phillips described, under the name of " mutants," 2 rats of the plus-selection series of extremely high grade. They proved to be heterozygotes between the average condition of the plus-selected race at that time, about + 3.75, and a new condition, not previously known in our hooded races, but resembling that seen in " Irish " rats, which are black all over except for a white spot on the belly and would be classed on our grading scale as about + 5½. In later generations we secured animals homozygous for the darker condition just described (that of Irish rats). The homozygous " mutant " race proved to be very stable in color pattern, varying only from 5¼ to 5¾, with a majority of animals graded 5½. Attempts to alter the modal condition of the race

by selection have thus far proved futile because of our inability to increase the race sufficiently to afford a basis for selection. Its inbredness and its feebleness are perhaps causally related.

The suggestion was made that the change from our plus-selected race, which had occurred in the mutant stock, might be due to some supplementary modifying factor, not to a change in the hooded factor itself.[4] If so, a cross with a race lacking the hooded factor or its "modifiers" might serve to demonstrate their distinctness by separating one from the other. A wild race seemed best suited for a test of this hypothesis, since it would be free from suspicion on the possible ground of harboring either the hooded pattern or its supposed modifier, which had converted the hooded pattern into the mutant. It was to be expected, if the hypothesis were correct, that the mutant character was hooded plus modifier; that then a cross with wild should produce in F_2 *hooded* young (lacking the modifier) as well as mutants and selfs. But if the mutant race had arisen through a change in the hooded factor itself, then the cross should produce only mutants and selfs, without hooded young in F_2. Crosses have now been made on a sufficient scale to show beyond question the correctness of the latter alternative, which is entirely in harmony also with the results described in the preceding parts of this paper.

Six homozygous "mutant" females of grade $+5\frac{1}{2}$ were mated with wild males of the same race described in Part I. They produced 46 young, all gray like wild rats and of grades as follows:

Grade	$5\frac{1}{2}$	$5\frac{3}{4}$	$5\frac{7}{8}$	6
No.	1	15	7	23

Exactly half of the 46 F_1 rats bore no white spot, *i. e.*, were of grade $+6$. Seven more bore only a few white hairs (grade $5\frac{7}{8}$). The remainder were very similar to the mutant parent in grade.

Several matings were made of the F_1 rats, brother with sister, which produced 212 F_2 young. About a quarter of these were black (non-agouti), the rest being gray (agouti). Both sorts included about equal numbers of individuals with and without white spots. No difference was observed in this respect between the progeny of spotted and of unspotted parents. Table 158 shows the F_2 young grouped family by family according to grade. Three of the four families are descended from a single mutant grandparent; the fourth family is descended from two different mutant grandparents which were bred simultane-

[4] This also is MacDowell's view. He says, p. 734: "The newly discovered factor acts independently of the other factors, is not modified by them, and does not modify them. Being the one difference between the mutant and the plus race at the time the mutant appeared, this factor affords a crucial test for the interpretation of the modifications that result from crosses."

ously to the same wild male in the same cage. The 10 F_2 young of this family may have been produced either by full brother and sister, or by half-brother and half-sister; it is uncertain which. All other F_2 young were produced by brother-sister matings.

TABLE 158

Table 158 shows the classification of the F_2 young obtained by crossing homozygous "mutant" with wild rats.

Mutant grandparents.	Grade of offspring.						Totals.
	5	$5\frac{1}{4}$	$5\frac{1}{2}$	$5\frac{3}{4}$	$5\frac{7}{8}$	6	
♀ 0630, $+5\frac{1}{2}$	...	3	12	9	2	20	46
♀ 0698, $+5\frac{1}{2}$	1	2	22	29	1	59	114
♀ 0694, $+5\frac{1}{2}$	...	...	12	11	...	19	42
♀ 0630, $+5\frac{1}{2}$, or 0636, $+5\frac{1}{2}$	...	1	3	1	...	5	10
Total	1	6	49	50	3	103	212

It will be observed that the F_2 young (table 158) which are white-spotted are in no case hooded. Their range of variation does not fall beyond that of the uncrossed mutant race. It is certain, therefore, that the " mutant " condition is not *hooded plus an independent Mendelian modifier*. It is a changed form of white-spotting, alternative to the form of spotting found in the race from which it was derived (the plus-selection series, generation 10). It is, without much doubt, also alternative to the self condition of wild rats, though fluctuation in grade obscures the segregation, which may, very likely, be imperfect. This serves to confirm the general conclusion that throughout the entire series of experiments with the hooded pattern of rats we are dealing with quantitative variations in one and the same genetic factor.

CONCLUSION

I have now presented the evidence which has led me to reject the hypothesis formerly held tentatively that modifying factors were largely concerned in changes produced in the hooded pattern of rats under repeated selection. This evidence seems to me to admit of only one consistent interpretation, that a single variable genetic factor was concerned in the original hooded race, that a changed condition of this same factor was produced in the minus race, and another changed condition in the plus race, and a third appeared in the mutant race. All are allelomorphs of each other, and of the non-hooded or self condition found in wild rats, yet all tend to modify each other in crosses. The character has a high degree of genetic stability, yet is sub-

ject to continuous genetic fluctuation. I have been unable to produce or to discover any race of spotted animals which is free from genetic fluctuation, though I have made an extended search. If MacDowell or any one else has discovered such a race, let it be produced.

It does not, of course, follow that because white spotting in rats is capable of indefinite modification through selection, therefore all heritable characters are equally capable of modification. Physiological limitations no doubt often limit the modifiability of characters. A sugar-beet can not be produced which is *all* sugar or much over 25 per cent. sugar. There has to be retained a plant mechanism for the production of sugar, a beet. Neither is it to be expected that the thorax of *Drosophila* can be decorated with an indefinite number of extra bristles. The bristles have to be attached to something, and the thorax of *Drosophila* is finite in size. It is not necessary to suppose that hypothetical modifying factors have been used up simply because variation ceases to progress in a particular direction. For no one, I suppose, would contend that variation is equally easy in all directions and in all characters. De Vries has taught us the significance of one-sided variation and we have become familiar with recurrent types of variation which are encountered first in one species and then in another. Such cases show that different kinds of germplasm are similar in structure and likely to undergo similar changes. But what happens to these spontaneous variations when once they have put in an appearance depends on external agencies, man or other factors in the struggle for existence. The modern study of evolution has indeed emphasized the importance of spontaneous internal changes in producing variations, but we still have to reckon with selection, natural and artificial, in determining the survival of variations as well as in controlling their magnitude and the direction of their further variation.

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